J. Membrane Biol. 73, 39–49 (1983)

The Journal of
Membrane Biology

pH Modulation of Glucose-Induced Electrical Activity in B-Cells: Involvement of Na/H and HCO_3/Cl Antiporters

Caroline S. Pace and John T. Tarvin*

Department of Physiology and Biophysics and Diabetes Research and Training Hospital
University of Alabama in Birmingham, Birmingham, Alabama 35294

Summary. Regulation of intracellular pH is an essential function and may be especially significant in the B-cell in which the influence of glucose on electrical activity is modulated by alterations in pH. Two possible regulatory processes have been examined: Na/H and HCO_3/Cl exchange, by using inhibitors, an ionophore, and changes of ionic concentrations. In the presence of 11.1 mM glucose we found that DIDS, an inhibitor of anion exchange, elicited a dose-response increase in the relative duration of the active phase with an ED_{50} of 99 μM. Probenecid (0.5 mM), an inhibitor of anion fluxes, also augmented the electrical activity (EA) due to glucose. Withdrawal of HCO_3^- elicited constant spike activity followed by a resumption of burst activity with a greater duration of the active phase compared to control. These data are consistent with predicted cellular acidification. However, reduction of Cl_o^- by isethionate substitution produced no marked effect on EA. In contrast, SO_4^{--} substitution for Cl^- resulted in variable effects characterized by constant spike activity or a decrease in the duration of the active and silent phases along with silent hyperpolarization. Tributyltin, a Cl/OH, ionophore enhanced EA at 0.25 μM with 120 mM Cl_o^-, but reduced EA with 10 mM Cl^- as would be predicted with either cellular acidification or alkalinization, respectively. Amiloride at 100 μM elicited constant spike activity perhaps due to inhibition of Na/H exchange. Reduction of Na_o^+ from 142.8 to 40.8 mM had a similar effect and enhanced the influence of amiloride. It appears therefore that interference with putative pH regulatory mechanisms in the B-cell are consistent with the hypothesis that cell pH is involved in regulation of EA.

Key Words DIDS · probenecid · amiloride · isethionate · tributyltin · mouse islet

Introduction

The characteristic electrical response of the B-cell to glucose involves at least both changes in K^+ and Ca^{++} conductances [2]. We have postulated that the membrane K^+ conductance is particularly sensitive to changes in H^+ concentration and have provided evidence for this by performing maneuvers designed to alter intracellular pH (pH_i) such as addition of permeable weak acids and weak bases [18]. Such data raise the possibility that the cyclical electrical activity (EA) of the B-cell is related to interactions between cell metabolism, pH, and K^+ conductance. An alternate approach to this hypothesis in the intact cell is to investigate the possible role of antiporters in the plasma membrane which may regulate pH_i. Of several such systems, the best studied are the Na/H and HCO_3/Cl antiporters found in invertebrate preparations such as squid axon and barnacle muscle [14].

However, there is some evidence in snail neurons that acid extrusion may occur by the influx of $NaCO_3^-$ in exchange for Cl^- efflux thereby excluding the participation of a Na/H exchange system [19]. In these studies we have examined the hypothesis that Na/H and HCO_3/Cl antiporters exist in the B-cell plasma membrane. Evidence for the presence of these exchangers has been obtained by 1) the application of inhibitors of anion transport such as DIDS (4,4′-diisothiocyano-2,2′-stilbene disulfonic acid) and probenecid; 2) the withdrawal of extracellular HCO_3^- or Cl^-; 3) the use of amiloride, an inhibitor of Na/H exchange; and 4) the decrease of $[Na^+]_o$. We have also studied the influence of a Cl/OH exchanger, tributyltin (TBT) [16], which should influence pH_i without directly affecting the activity of native Na/H or HCO_3/Cl antiport systems.

Our results suggest that both Na/H and HCO_3/Cl antiporters exist in the B-cell membrane, and that they are intricately involved in the regulation of the oscillatory nature of EA presumably by controlling pH_i.

Materials and Methods

Islets of Langerhans were microdissected from fed CBA/J retired-breeder male mice (Jackson Laboratories, Bar Harbor,

* *Present address:* Department of Physics, University of Mississippi, Oxford, Miss. 38677.

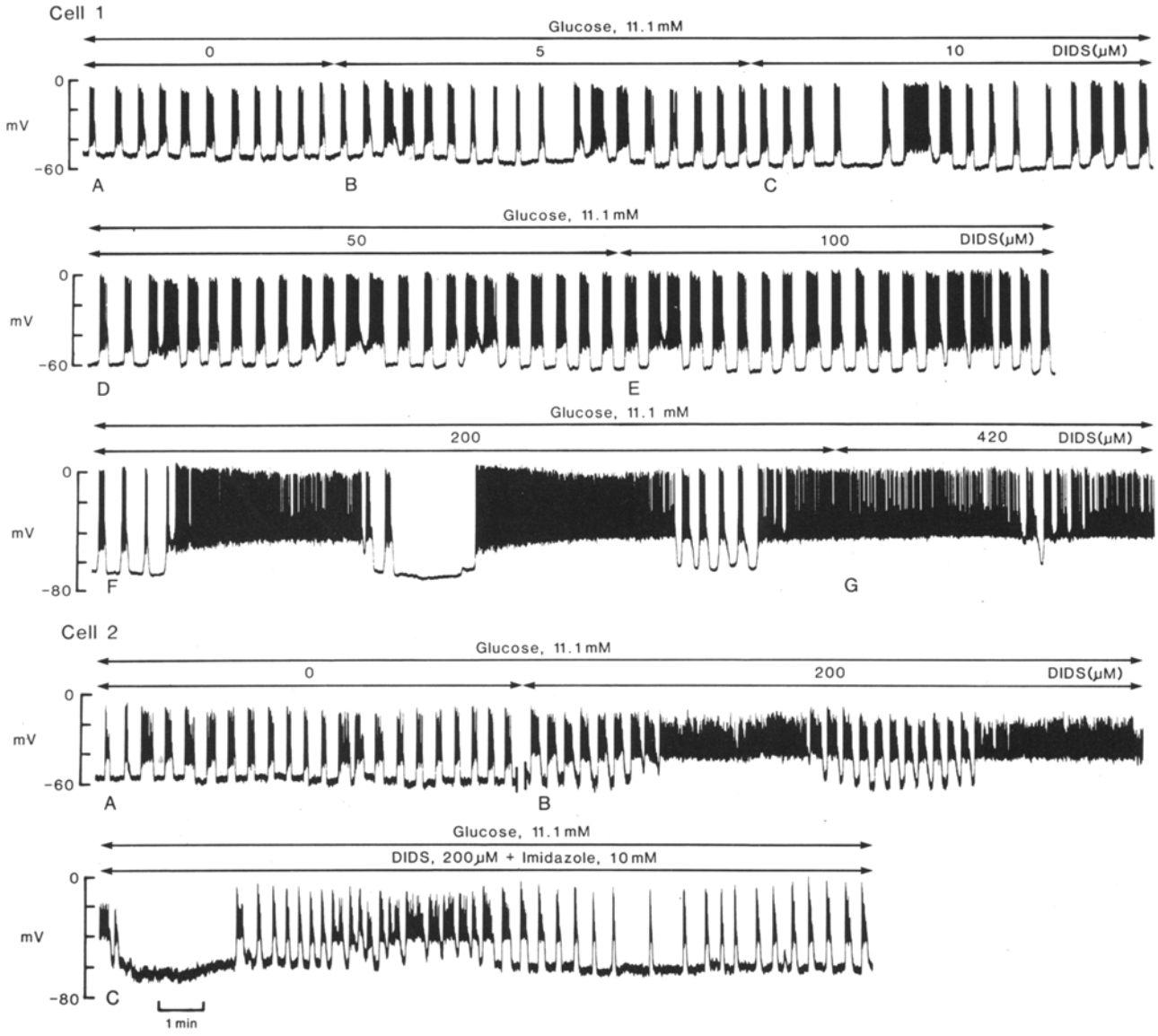

Fig. 1. Effect of DIDS on glucose-induced electrical activity. Cell 1: (*A*) Control activity in the presence of 11.1 mM glucose. (*B–G*) Effects of the addition of 5, 10, 50, 100, 200, and 420 μM DIDS to a medium containing 11.1 mM glucose. All records are continuous. Cell 2: (*A*) Control activity in the presence of 11.1 mM glucose. (*B*) Addition of 200 μM DIDS. (*C*) Addition of 10 mM imidazole to a medium containing 200 μM DIDS and 11.1 mM glucose. Records *B* and *C* are continuous

Maine). Microelectrode recordings from single B-cells were obtained using standard electrophysiologic techniques, as described in detail elsewhere [10]. The ionic content of the modified Krebs-Henseleit perifusion medium was (mM): Na^+, 136.2; K^+, 5; Ca^{++}, 2.5; Mg^{++}, 1.2; Cl^-, 120; HCO_3^-, 25; SO_4^{--}, 1.2; $H_2PO_4^-$, 1.2. The medium was buffered with 16 mM HEPES, and the pH was adjusted to 7.4 by the addition of NaOH. The flow rate was 2.0 ml/min, and the volume of the chamber was about 250 μl. The time required for total exchange of solutions was <5 sec. Glucose was added to the above medium just before perifusion, and the medium was equilibrated with 95% O_2, 5% CO_2 for a final pH of 7.4. DIDS was added from a freshly mixed stock solution. Older stocks seemed to lose their potency. TBT was also added from a stock solution, but showed no loss of potency with time. For the studies without HCO_3^-, the following medium was used (mM): Na^+, 130; K^+, 5.9; Ca^{++}, 2.6; Mg^{++}, 1.2; Cl^-, 139.8; SO_4^{--}, 1.2; $H_2PO_4^-$, 1.2. The medium was buffered with 20 mM HEPES, and the pH was adjusted to 7.4 by the addition of NaOH. The solution was not gassed.

Chemicals: HEPES was obtained from Research Organics, Cleveland, Ohio; DIDS from Calbiochem-Behring Corp., La Jolla, Calif.; TBT from Alfa Products, Danvers, Mass.; probenecid and sodium isethionate from Sigma, St. Louis, Mo.; and amiloride from Merck Sharp & Dohme, Westpoint, Pa.

Results

Our hypothesis is that pH_i is an important determinant of the pattern of glucose-induced EA in the

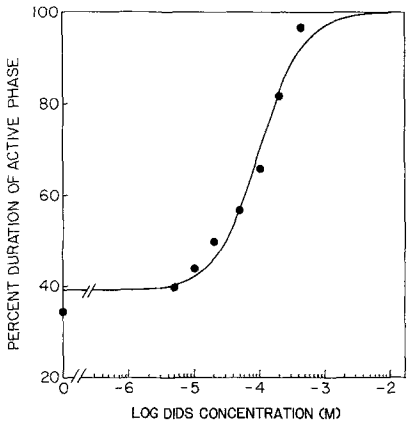

Fig. 2. Percent electrical activity as a function of the external DIDS concentration. The data are mean values from six islets; the standard errors are less than 5% for all data points except 420 μM, which was obtained for only one cell. All data were obtained in normal Cl^- media containing 11.1 mM glucose. The smooth curve was drawn to fit the equation:

$$\%EA = EA_o + \frac{100 - EA_o}{1 + (D/ED_{50})^b}$$

where %EA is the percent electrical activity at any external DIDS concentration D; EA_o is the control electrical activity in the absence of DIDS; ED_{50} is the effective dose of DIDS resulting in a response half way between EA_o and 100% activity; and b is the slope-factor of the logit-log plot. Least-squares curve fitting gave final parameter estimates of M ± SE of: $EA_o = 39 \pm 3$, $ED_{50} = 99 \pm 15$ μM, $b = 1.3 \pm 0.2$

B-cell. We have previously shown that alteration of extracellular pH (pH_o) has a profound effect on electrical activity [18]. In fact, alteration of pH_o has been shown to alter pH_i in B-cells [7]. We have also shown that a permeable weak base or acid affects EA as predicted based on results obtained by altering pH_o. Since alteration of pH_i in the B-cell influences the EA, it seems reasonable to assume that there is a pH_i regulatory mechanism in the B-cells. This assumption has been tested by exposing the B-cells to various manipulations that have been found to influence acid extrusion in other types of cells. In designing our experiments we have examined the hypothesis that HCO_3/Cl and Na/H antiport systems are important regulatory systems for maintenance of pH_i. Hence, we have attempted to inhibit these antiport systems by use of appropriate pharmacological agents or substitution of the pertinent ion in the extracellular medium, and monitor EA to determine the influence of these conditions on alteration of pH_i.

Sensitivity of Electrical Activity to Inhibition of HCO_3/Cl Exchange

DIDS is an effective inhibitor of HCO_3/Cl exchange in red blood cells [6] and has been used to obtain evidence for the role of HCO_3/Cl exchange in regulation of pH_i in invertebrate neurons and muscle [14]. The application of 0 to 420 μM DIDS to a medium containing 11.1 mM glucose induced a dose-related increase in EA (Figs. 1 and 2). DIDS increased the fraction of time during which the electrical response was in the active phase. The effective dose resulting in a half-maximal response (ED_{50}) was calculated to be 99 ± 15 μM (Fig. 2). These experiments were conducted with minimal light due to the light-sensitivity of DIDS. A solution of 50 μM DIDS which was exposed to light for 1 hr before use had no influence on EA. Prolonged exposure of cells to solutions containing more than 100 μM DIDS produced irreversible effects on the EA (*data not shown*).

In order to determine if the influence of DIDS, which may decrease pH_i, could be antagonized by a permeant weak base, we added 10 mM imidazole to a preparation previously exposed to 200 μM DIDS plus 11.1 mM glucose (Fig. 1, Cell 2). Imidazole immediately induced a silent hyperpolarization of 2.5-min duration followed by resumption of burst activity with a marked decrease in the duration of the active phase.

Probenecid has been used to inhibit fluxes of anions (Cl^- and OH^-) in a wide variety of cells [11]. We found 0.5 mM probenecid to augment the duration of the active phase in the presence of 11.1 mM glucose (Fig. 3). However, 10 mM probenecid provoked an initial burst of spikes followed by silent hyperpolarization. The effect of probenecid was also dose-related (0.1 to 0.5 mM) as observed in three different cells. In contrast to DIDS the influence of probenecid on EA was reversible. Since the influence of DIDS and probenecid on the EA is similar to the effects of a decrease in pH, then it may reasonably be assumed that a HCO_3/Cl antiport system is involved in regulation of pH_i in the B-cells.

Requirement for Extracellular HCO_3^- and Cl^-

Since in squid axons, snail neurons, and barnacle muscle the regulation of pH_i is dependent on $[HCO_3^-]_o$ (5), we examined the influence of HCO_3^- withdrawal on the EA. If there is a coupled HCO_3/Cl exchange present in the B-cell, then we would anticipate that extracellular HCO_3^- would be involved in the regulation of pH_i. Therefore, removal of extracellular HCO_3^- would be expected to decrease pH_i, and increase EA. Indeed, as shown in Fig. 4, withdrawal of medium HCO_3^- initially produced depolarization to the plateau phase and constant spike activity of about 4-min duration, com-

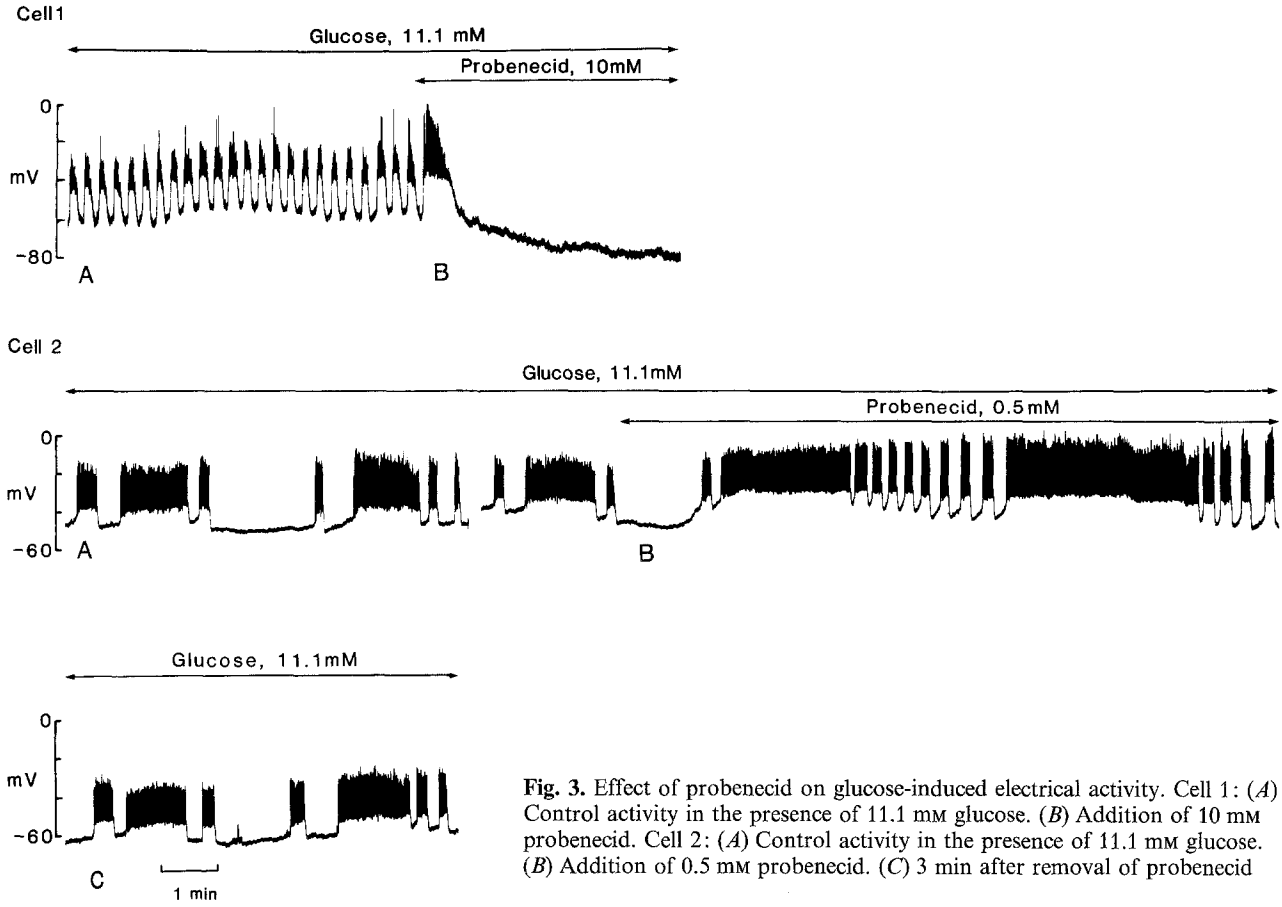

Fig. 3. Effect of probenecid on glucose-induced electrical activity. Cell 1: (*A*) Control activity in the presence of 11.1 mM glucose. (*B*) Addition of 10 mM probenecid. Cell 2: (*A*) Control activity in the presence of 11.1 mM glucose. (*B*) Addition of 0.5 mM probenecid. (*C*) 3 min after removal of probenecid

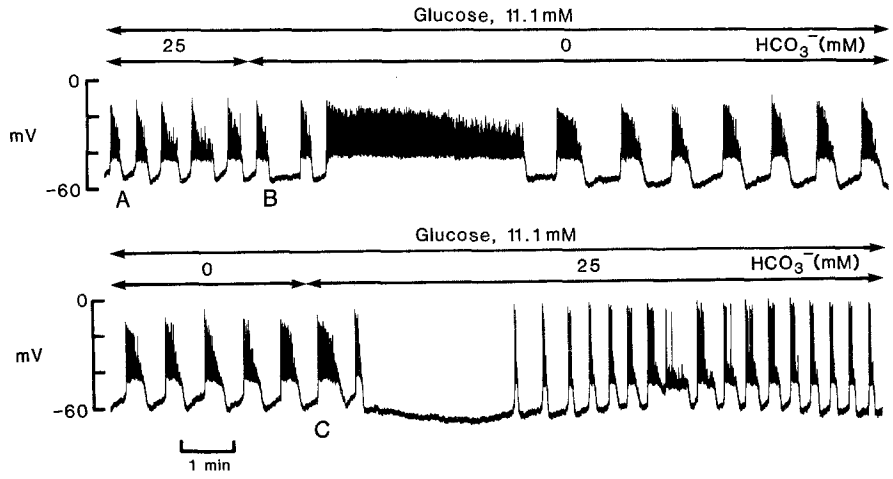

Fig. 4. Effect of bicarbonate withdrawal on glucose-induced electrical activity. (*A*) Control activity in the presence of 11.1 mM glucose and 25 mM HCO_3^-. (*B*) Removal of HCO_3^-. (*C*) Readdition of 25 mM HCO_3^-

pared to about a 20-sec plateau duration in the presence of HCO_3^-, after which burst activity resumed with a marked increase in the active phase duration compared to control (Fig. 4*A* and *B*). Upon reintroduction of a HCO_3^--containing medium (Fig. 4*C*) there was an initial 2.5-min silent hyperpolarization followed by resumption of a pattern of EA similar to the initial control activity. Similar effects were observed in three other cells.

Since it has been shown that extracellular HCO_3^- exchanges for intracellular Cl^- in invertebrate preparations [14], one would not expect a pronounced effect on the EA when extracellular Cl^- is substituted by SO_4^{--}, unless the concentra-

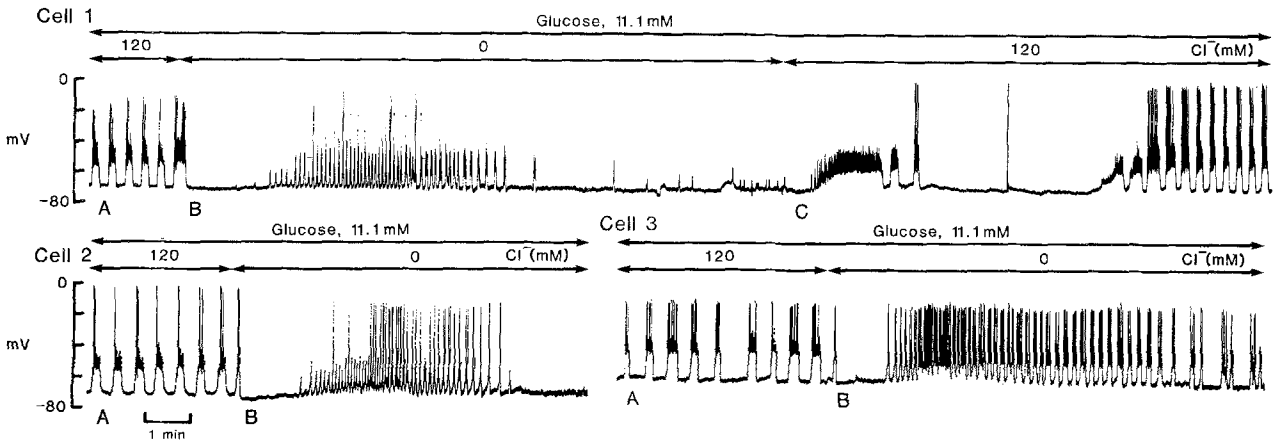

Fig. 5. Effects of total medium Cl^- replacement by SO_4^{--} on glucose-induced electrical activity. Cell 1: (*A*) Control activity in the presence of 11.1 mM glucose. (*B*) Replacement of medium Cl^- by SO_4^{--}. Total Na^+ was maintained constant, sucrose being used to maintain medium osmolarity. (*C*) Reintroduction of Cl^- medium. Cell 2 and 3: (*A*) Control activity in the presence of 11.1 mM glucose. (*B*) Replacement of medium Cl^- by SO_4^{--}

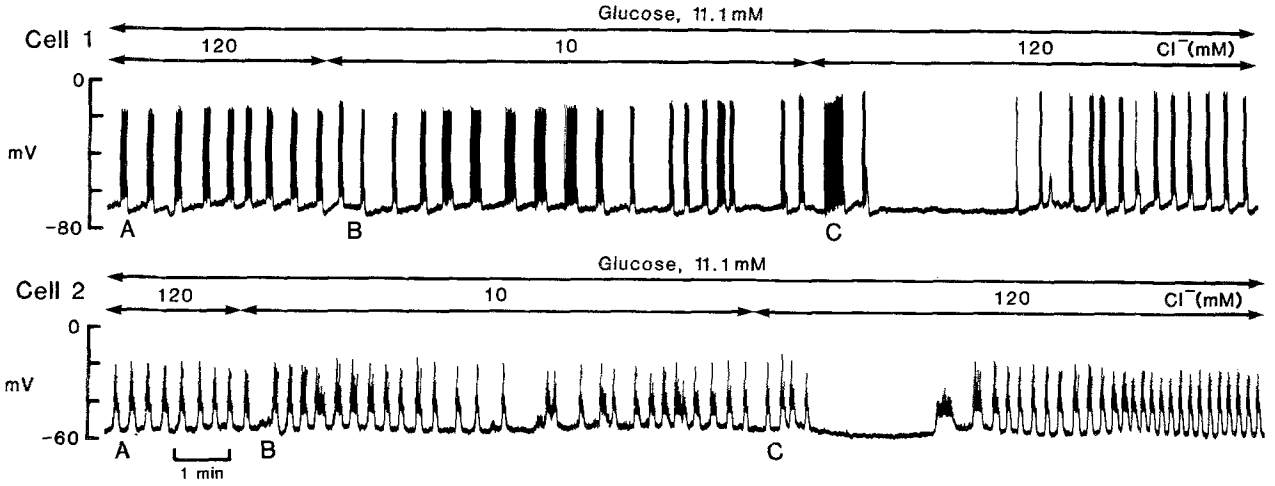

Fig. 6. Effect of partial medium Cl^- replacement by isethionate on glucose-induced electrical activity. Cell 1: (*A*) Control activity in the presence of 11.1 mM glucose. (*B*) Partial replacement of medium Cl^- by isethionate. (*C*) Reintroduction of normal Cl^- medium. Cell 2: Same as Cell 1

tion of intracellular Cl^- is substantially reduced after efflux of Cl^- down its concentration gradient. The effect of total replacement of Cl_o^- by SO_4^{--} is shown in Fig. 5. Cl^- replacement with SO_4^{--} produced variable effects characterized by increased burst frequency with a marked decrease in the duration of active and silent phases, or transient depolarization to the active phase with a return to burst activity of greater frequency than observed in the presence of Cl^- (Fig. 5). This activity was preceded by a brief silent period. As shown in Fig. 5, Cell 1, after a 6-min train of bursts there was a loss of oscillatory activity and only intermittent bursts. Upon reintroduction of Cl^- there was a brief burst of constant spike activity followed by a 4-min silent hyperpolarization, after which there was resumption of control burst activity. Cells 2 and 3 show similar results. These electrical events are different from those observed with DIDS and probenecid. It is possible that SO_4^{--} has an effect on the EA independent of Cl^- substitution.

Na isethionate was also used to replace NaCl. In these experiments 10 mM Cl^- was present in the form of KCl and $CaCl_2$ in contrast to total replacement of Cl^- with SO_4^{--}. There was no marked effect on EA upon reduction of Cl^- from 120 to 10 mM with isethionate (Fig. 6). There was a modest increase in the duration of the active phase in four cells impaled in different islets. Upon return to normal Cl^-, EA continued for about 1 min and was followed by a silent hyperpolarization of 2

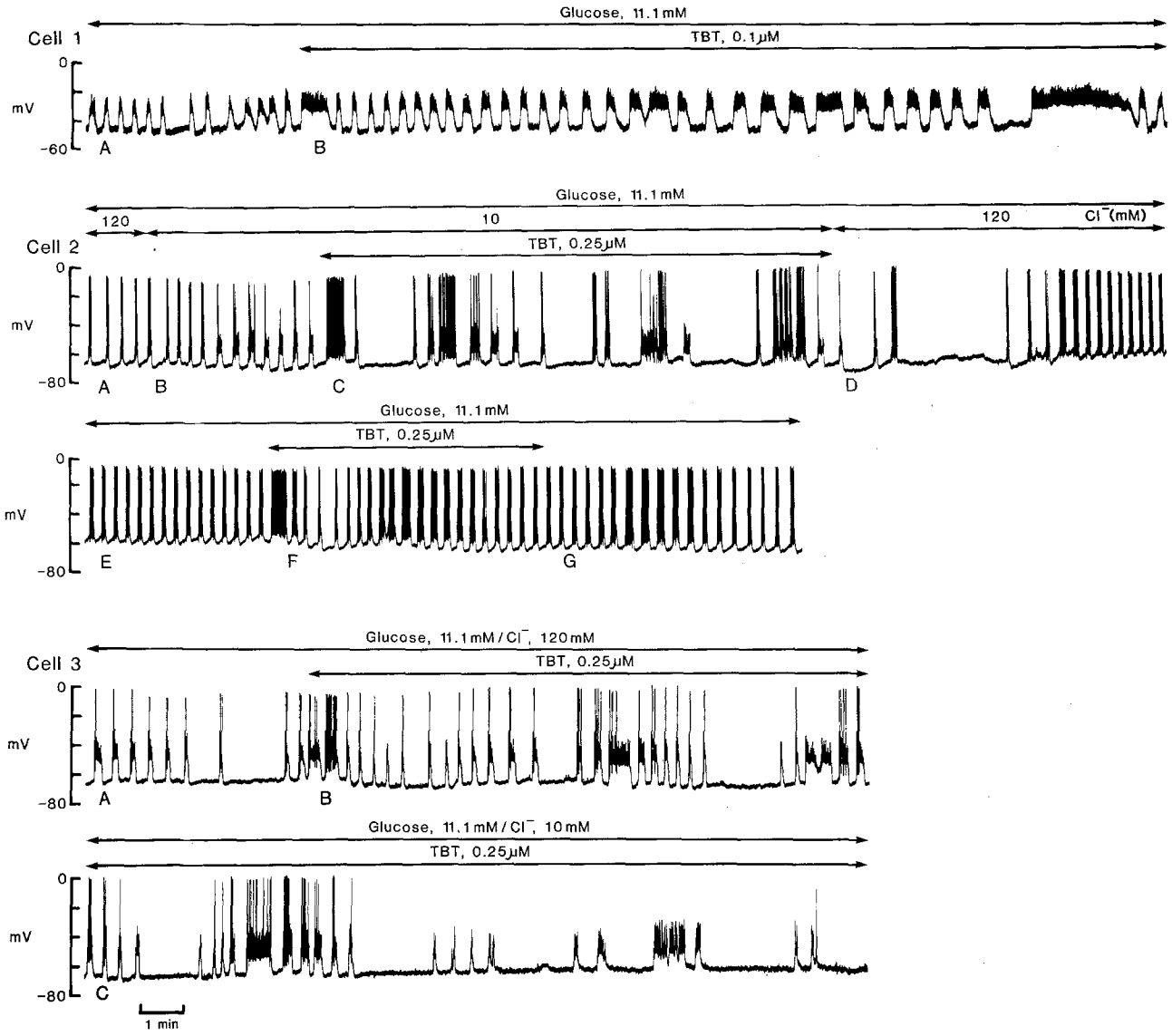

Fig. 7. Effect of TBT in both normal and reduced Cl^- media on glucose-induced electrical activity. Cell 1: (*A*) Control activity in the presence of 11.1 mM glucose. (*B*) Addition of 0.1 μM TBT. Cell 2: (*A*) Control activity in the presence of 11.1 mM glucose. (*B*) Partial replacement of medium Cl^- by isethionate. (*C*) Addition of 0.25 μM TBT. (*D*) Removal of TBT and reintroduction of normal Cl^- medium. (*E*) Control activity in the presence of 11.1 mM glucose. (*F*) Addition of 0.25 μM TBT. (*G*) Removal of TBT. *D* and *E* are separated by 28 min. Cell 3: (*A*) Control activity in the presence of 11.1 mM glucose. (*B*) Addition of 0.25 μM TBT. (*C*) Partial replacement of medium Cl^- by isethionate in the presence of 0.25 μM TBT. Record is continuous. In all cells, medium Cl^- is 120 mM except as noted

to 3 min before resumption of control EA of slightly augmented burst frequency.

Sensitivity to Anion Transport Ionophore

Tributyltin (TBT) at low concentrations has been shown to function as a Cl/OH anion exchanger in mitochondria, red blood cells, artificial membranes [16], and gastric plasma membrane vesicles [12]. We have used TBT in these experiments in an effort to determine whether a more direct method of altering pH_i by exchange of Cl_o^- for OH_i^- would increase EA in a manner similar to that induced by inhibition of HCO_3/Cl exchange. We found that 0.1 μM TBT plus 11.1 mM glucose immediately increased the relative duration of the active phase (Fig. 7, Cell 1). In fact, the effect of TBT appeared to increase with time in contrast to the application of DIDS, probenecid, or the withdrawal of medium HCO_3^-. The increase in EA is consistent with the exchange of Cl_o^- with OH_i^-. In Fig. 7, Cell 2, reduction of Cl_o^- from 120 to

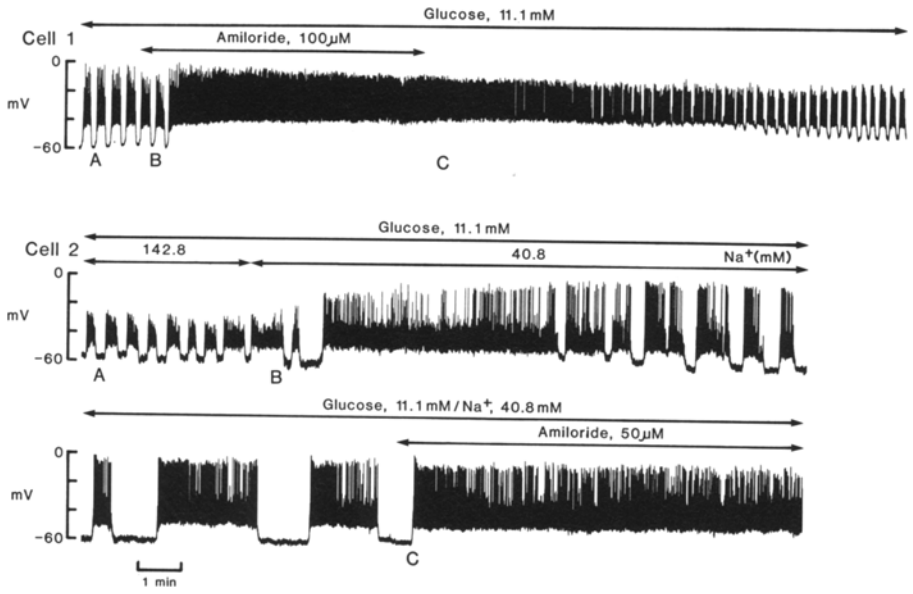

Fig. 8. Effect of amiloride in both normal and reduced Na^+ media on glucose-induced electrical activity. Cell 1: (*A*) Control activity in the presence of 11.1 mM glucose. (*B*) Addition of 100 μM amiloride. (*C*) Removal of amiloride. Cell 2: (*A*) Control activity in the presence of 11.1 mM glucose and normal medium Na^+. (*B*) Partial replacement of medium Na^+ by choline. (*C*) Addition of 50 μM amiloride in the presence of 11.1 mM glucose and reduced medium Na^+. Record is continuous. Medium Na^+ is 142.8 mM except as noted

10 mM with isethionate elicited little change in EA (*see also* Fig. 6). Subsequent addition of 0.25 μM TBT produced an irregular pattern of EA characterized by an overall reduction in burst frequency interrupted by long silent phases. This is in marked contrast to the increase in EA elicited by TBT in the presence of 120 mM Cl^- (Fig. 7, Cell 1). The withdrawal of TBT and reintroduction of 120 mM Cl^- produced an initial silent period followed by regular burst activity. Subsequent addition of TBT to the same cell with normal Cl^- enhanced EA similar to that seen previously (Fig. 7, Cell 1).

Sensitivity to Inhibition of Na/H Exchange

We have previously found that acceleration of Na/H exchange induced by monensin inhibited EA, as would be predicted if alkalinization occurred [10]. This suggested the possibility that a native Na/H exchanger may function to regulate pH_i. To test this hypothesis we used amiloride, which has been reported to inhibit Na/H exchange in both cellular and epithelial systems [4]. In addition we decreased $[Na^+]_o$ in an effort to decrease the rate of Na/H exchange.

The addition of 100 μM amiloride to a medium containing 11.1 mM glucose immediately provoked depolarization to the plateau level and constant spike activity (Fig. 8, Cell 1). Upon removal of the drug, the cell continued to display constant spike activity for 7 min followed by a gradual return to oscillatory EA, although of greater burst frequency than control. In an additional experiment we decreased $[Na^+]_o$ to 40.8 mM, which in itself elicited constant spike activity at the plateau level and a gradual return to burst activity with a marked increase in the relative duration of the active phase (Fig. 8, Cell 2). Addition of 50 μM amiloride to the low Na^+ medium again elicited constant spike activity similar to that obtained in the presence of normal $[Na^+]_o$. Similar results with low Na^+ with and without amiloride were obtained in two other cells. The effect of amiloride was reversible within 2 min upon return to a normal Na^+ medium (*data not shown*).

Discussion

It has been amply demonstrated in invertebrate nerve and muscle cells that the pH_i regulatory mechanism primarily consists of Na/H exchange in association with HCO_3/Cl exchange [14]. Because of our recent report that pH influences glucose-induced EA [18], we were interested in determining whether there existed similar endogenous ionic antiport systems in the plasma membrane to regulate pH_i in addition to Ca^{++} buffering mechanisms possibly involving Ca/H exchange in the secretory granule membrane [9]. In fact, it is possible that the extrusion of H^+ from the B-cell is very efficient; this is indicated by the finding that glucose produced a dose-related increase in the net output of H^+, even though ^{14}C-DMO (5,5′-dimethyloxazolidine-2,4-dione) failed to reveal any change in the steady-state pH level of the cell [7, 17]. Two conclusions may be derived from such observations: firstly, that efficient mechanisms exist in the membrane of this cell for extrusion

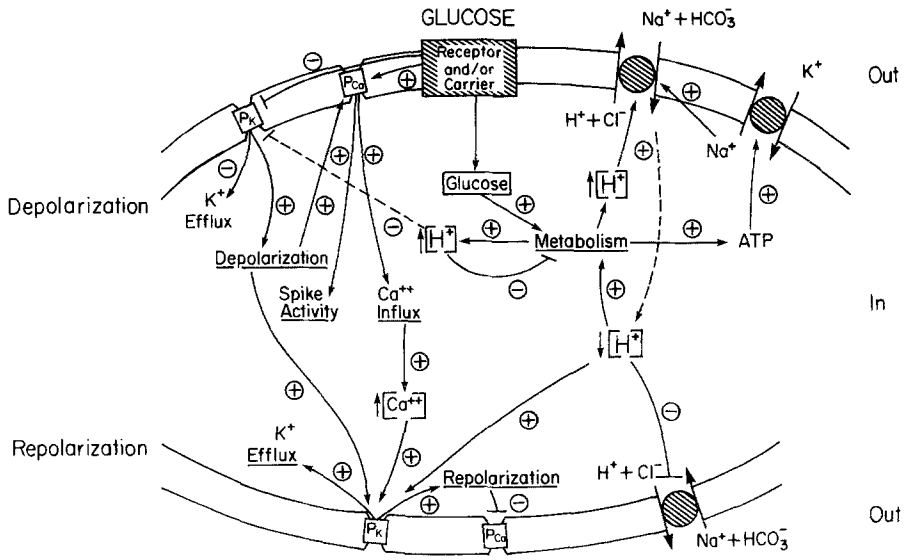

Fig. 9. Model to account for the interaction of H^+ and anion transport on the mechanisms controlling glucose-induced EA. Glucose decreases P_K initiating depolarization and an increase in the concentration of intracellular Ca^{++}. These events in turn activate P_K and initiate repolarization of the membrane potential. The generation of H^+ may modulate P_K in the following manner. The metabolism of glucose results in an increased intracellular concentration of H^+, which in turn may serve as a feedback inhibitor of metabolism via the activity of phosphofructokinase [20]. It is possible that H^+ generated by glycolysis in the B-cell is transported out of the cell via coupled Na/H and HCO_3/Cl exchange systems. The increase in pH may then serve to increase the rate of glycolysis as occurs in yeast and ascites cells [13]. The influence of glucose on P_K and metabolism may be coupled by $[H^+]_i$, since an increase in $[H^+]_i$ may lead to an increased Na/H and/or HCO_3/Cl exchange. This increased exchange will serve to decrease $[H^+]_i$ and, in turn, increase P_K. The driving force for the coupled antiporter system is probably due to the gradient of Na^+ which is maintained by the activity of a Na/K pump

of H^+, and secondly, that oscillations of pH_i may occur so that no change in steady-state pH is detectable by radioactive tracer techniques. It is conceivable that small and rapid changes in pH_i may interact with the K^+ channel to influence the oscillatory pattern of electrical activity by decreasing P_K as occurs in neural tissue [22].

Gradient driven antiporters such as Na/H and HCO_3/Cl exchange would be effective in back regulation of cell pH following alterations in the glucose metabolism. Alterations of the activity of these transport systems should have predictable effects on pH_i regulation and in turn on EA. Indeed, if the pH coupling hypothesis is correct, measurement of the nature of EA in the B-cell would be a monitor of the direction of change of pH_i (*see* model in Fig. 9).

HCO_3/Cl Antiport

Several lines of evidence in various cell types have shown that pH_i is maintained by uptake of HCO_3^- and efflux of Cl^- [14]. We have obtained evidence for the existence of a HCO_3/Cl antiporter in the B-cell membrane by the use of DIDS. Inhibition of anion exchange by this agent would be expected to decrease pH_i resulting in either bursts with a longer active phase or the induction of depolarization and constant spike activity, as was found to occur when pH_o or pH_i was decreased by altering the pH of the medium or by using a permeable weak acid, respectively [18]. As documented in Fig. 1, DIDS produced these effects. Probenecid, which may also inhibit anion transport [11], produced similar effects at low concentrations. Conversely, at a high concentration, the drug inhibited EA. The reason for this inhibition is not known, but may reflect nonspecific interference with other cellular parameters.

An alternative method of perhaps modifying pH_i by altering anion flux via the antiporter is to change the medium concentration of HCO_3^- or Cl^-. In the B-cell removal of HCO_3^-, at constant extracellular pH, resulted in initial depolarization and constant spike activity followed by resumption of the burst pattern. Whereas the former effect was reminiscent of the effect of DIDS, the resumption of burst activity was unexpected. Moreover, reintroduction of HCO_3^- resulted in a transient quiet phase. Presumably, in terms of the pH regulatory mechanism, the alteration in pH_i in the case of HCO_3^- removal can be reversed more readily than in the presence of DIDS and hence when HCO_3^- is reintroduced there is a transient alkalinization.

If, for example, there is an association between the Na/H and HCO_3/Cl exchange physically in the membrane, then an inhibitor of anion exchange may modify the ability of the Na/H exchanger to compensate on its own, whereas this would be unimpaired with HCO_3^- removal alone.

When Cl_o^- was reduced by SO_4^{--} substitution, there were bursts of activity preceded and followed by prolonged silent periods. This electrical response was markedly different from that due to manipulations predicted to inhibit HCO_3/Cl exchange. According to results obtained in snail neurons, withdrawal of Cl_o^- should have no influence on acid extrusion [19]. However, since the B-cell is much smaller than the snail neuron, changes in ionic gradients would be expected to induce more rapid alterations in the transmembrane gradient of ions. It is conceivable that the removal of Cl_o^- results in an exit of Cl_i^- and therefore initially increases the activity of the antiport systems leading to a transient increase in pH_i. But upon a decrease in $[Cl^-]_i$ below a threshold level, there may be an inhibition of anion exchange and a consequent decrease in pH_i, which should result in an increase in EA. The reason for the subsequent silent phase is not understood. Further studies are required to explain the effects of SO_4^{--} substitution of Cl^-. Readdition of Cl_o^- elicits regular burst activity only after several minutes, which is probably due to re-equilibration of the Cl^- gradient.

In contrast to SO_4^{--}, isethionate substitution for Cl^- had much less effect on EA, resulting in irregular patterns, but clearly no inhibition. Isethionate, a monovalent anion, may be more effectively transported than SO_4^{--}.

Interestingly, in sheep-heart Purkinje fibers, a Cl^--free solution elicits an increase in pH_i [21]. This is presumably due to the enhanced entry of HCO_3^- dependent on the enhanced Cl^- gradient. It should be noted that this reversible anion antiporter probably is not coupled to Na/H exchange, but is postulated to be responsible for maintaining a high level of $[Cl^-]_i$ [21]. The extent to which this system is involved in regulation of pH_i under normal conditions is not clear.

Cl/OH Ionophore

The trialkyltin compounds have been shown to inhibit oxidative phosphorylation in mitochondria in the absence of anions [8]. A second mode of action, revealed in the presence of Cl^-, has been characterized as Cl/OH exchange [16]. This anion ionophoric effect also occurs in erythrocytes and liposomes. Mitochondrial inhibitors and uncouplers, when applied in the presence of glucose, hyperpolarize the B-cell membrane, presumably via the release of Ca^{++} from mitochondria and consequent activation of P_K [2]. Since TBT, at the low levels used in this work, induced an increase in EA rather than hyperpolarization and a decrease in EA, we concluded that the primary mode of action of TBT in the intact B-cell is Cl/OH exchange.

We used TBT in this study to examine the influence of an alteration of pH_i independent of the HCO_3/Cl and Na/H exchange systems. In addition, we attempted to reverse the direction of exchange by reducing Cl_o^- to determine if we could achieve alkalinization and inhibition of EA. We found that the addition of low levels of TBT with $[Cl^-]_o = 120$ mM (*see* Fig. 7) prolonged the active phase of EA, similar to the results obtained by cellular acidification [18]. This result, if due to a decrease in pH_i, would require the exit of OH^- and the entry of Cl^-. Appropriate calculations indicate that this movement occurs only for $[Cl^-]_i <$ 48 mM, when $[Cl^-]_o$ is 120 mM. Conversely, for $[Cl^-]_o = 10$ mM, the effect of TBT was reversed, which would be expected if Cl^- exit and OH^- entry were occurring. In normal medium Cl^-, the effect of TBT was not as marked as that obtained by inhibition of HCO_3/Cl exchange with DIDS. This may indicate that the latter system, in combination with Na/H exchange, is more efficient in the regulation of pH_i. The finding, in normal medium Cl^-, that the intracellular Cl^- activity is probably less than 48 mM does not agree with the $^{36}Cl^-$ flux studies of Sehlin [15], in which Cl_i^- levels greater than 75 mM were reported. This discrepancy may be due in part to an intracellular compartmentalization of Cl^-, the nonspecific binding of Cl^- to intracellular components, or a reduced intracellular activity coefficient for Cl^-. Upon reduction of Cl_o^- to 10 mM, TBT decreased EA suggesting alkalinization of the intracellular space. This effect was independent of the order of application of TBT, e.g., before or after reduction of Cl_o^-. The influence of TBT was not immediately reversible, which may have been due to a delay in the release of TBT by the plasma membrane.

It should be noted that, unless HCO^3/Cl exchange is coupled to an energy source, the probable direction of exchange under normal conditions would be Cl^- in for HCO_3^- out, an effect which would tend to acidify the cell. Hence, the only way in which HCO_3/Cl exchange can assist in the regulation of pH_i is to reverse the intrinsic exchange direction. The most probable source for this is coupling to the Na^+ gradient, as found in

invertebrate systems [14]. The fact that amiloride, a blocker of Na/H exchange and DIDS, a blocker of HCO_3/Cl exchange, produced similar effects on EA strongly suggests that a similar system may be operating in the B-cell.

Na/H Antiport

It has been difficult to unequivocally document the presence of a Na/H antiporter by the mere reduction of Na_o^+ since it is possible that $NaCO_3^-$ enters the cells in exchange for Cl_i^- with no net movement of H^+ [3]. In addition, the effect of Na_o^+ removal on recovery from an acid load provides no evidence that Na/H exchange is coupled to HCO_3/Cl exchange [19]. Thus it is essential to use an inhibitor of Na/H exchange such as amiloride [1]. Either the addition of 100 μM amiloride or a reduction of Na_o^+ to 40.8 mM immediately resulted in constant spike activity very similar to that induced by the withdrawal of HCO_3^-, addition of DIDS greater than 200 μM, or application of the permeable weak acid glycodiazine [18]. At low Na_o^+, 50 μM amiloride was as effective as 100 μM amiloride at normal Na_o^+. Thus the effects of modification of the rate of Na/H exchange are consistent both with the presence of such an exchange in the plasma membrane and with a role for this system in pH regulation.

An essential question which remains to be answered is the extent to which the HCO_3/Cl and Na/H antiport systems are coupled. Since the application of a high concentration of amiloride is as effective as the application of a high concentration of DIDS, our results suggest that inhibition of one antiport system inhibits a putatively coupled system. If this were not so, one would predict that inhibition of HCO_3/Cl exchange would accelerate Na/H exchange and vice versa. Further studies are required to clarify this question. In addition, it would be desirable to actually measure changes in pH_i or $[Cl^-]_i$ using intracellular electrodes.

We have presented evidence that the control of pH_i profoundly influences the pattern of EA in the B-cell. In fact, inhibition of the antiport systems produces an effect similar to that obtained by increasing glucose above 16.7 mM. It is tempting to speculate that abnormalities in the B-cell result in either an excess H^+ production or a malfunction of the antiport systems. Such abnormalities may excite the plasma membrane independently of the prevailing level of extracellular glucose. This could lead to glucose insensitivity and impairment of the transfer of information from the plasma membrane to the secretory complex.

This study was supported by National Institutes of Health Grant AM-21973. C.S. Pace is a recipient of a Research Career Development Award AM-00499. J.T. Tarvin is supported, in part, by a Juvenile Diabetes Foundation Postdoctoral Grant. The authors gratefully acknowledge the helpful advice and discussion of Dr. R.A. Frizzell and Dr. G. Sachs during the course of this work.

References

1. Aickin, C.C., Thomas, R.C. 1977. An investigation of the ionic mechanism of intracellular pH regulation in mouse soleus muscle fibers. *J. Physiol. (London)* **273**:295–316
2. Atwater, I. 1980. Control mechanisms for glucose induced changes in the membrane potential of mouse pancreatic B-cell. *Clin. Biol. (Portugal)* **5**:299–314
3. Becker, B.F., Duhm, J. 1978. Evidence for anionic cation transport of lithium, sodium and potassium across the human erythryrocyte membrane induced by divalent anions. *J. Physiol. (London)* **282**:149–168
4. Benos, D.J. 1982. Amiloride: A molecular probe of sodium transport in tissues and cells. *Am. J. Physiol.* **242**:C131–C145
5. Boron, W.F., McCormick, W.C., Roos, A. 1981. pH regulation in barnacle muscle fibers: Dependence on extracellular sodium and bicarbonate. *Am. J. Physiol.* **240**:C80–C89
6. Cabantchik, Z.I., Knauf, P.A., Rothstein, A. 1978. The anion transport system of the red blood cell. The role of membrane protein evaluated by the use of probes. *Biochim. Biophys. Acta* **515**:289–302
7. Carpinelli, A.R., Sener, A., Herchuelz, A., Malaisse, W.J. 1980. Stimulus-secretion coupling of glucose-induced insulin release. Effect of intracellular acidification upon calcium efflux from islet cells. *Metabolism.* **29**:540–545
8. Dawson, A.P., Selwyn, M.J. 1975. The action of tributyltin on energy coupling in coupling-factor-deficient submitochondrial particles. *Biochem. J.* **152**:333–339
9. Pace, C.S., Sachs, G. 1982. Glucose-induced proton uptake in secretory granules of B-cells in monolayer culture. *Am. J. Physiol.* **242**:C382–C387
10. Pace, C.S., Tarvin, J.T., Smith, J.S. 1982. The role of protons in glucose-induced stimulus-secretion coupling in pancreatic islet B-cells. *In:* Intracellular pH: Its Measurement, Regulation, and Utilization in Cellular Functions. R. Nuccitelli and D.W. Deamer, editors. pp. 483–512. Alan R. Liss, New York
11. Pollard, H.B., Pazoles, C.J., Creutz, C.E., Zinder, O. 1979. The chromaffin granule and possible mechanisms of exocytosis. *Int. Rev. Cytol.* **58**:159–197
12. Rabon, E., Chang, H., Sachs, G. 1978. Quantitation of hydrogen ion and potential gradients in gastric plasma membrane vesicles. *Biochemistry* **17**:3345–3353
13. Rapp, P.E. 1979. An atlas of cellular oscillators. *J. Exp. Biol.* **81**:281–306
14. Roos, A., Boron, W.F. 1981. Intracellular pH. *Physiol. Rev.* **61**:296–434
15. Sehlin, J. 1978. Interrelationship between chloride fluxes in pancreatic islets and insulin release. *Am. J. Physiol.* **235**:E501–E508
16. Selwyn, M.J., Dawson, A.P., Stockdale, M., Gains, N. 1970. Chloride-hydroxide exchange across mitochondrial, erythrocyte and artificial lipid membranes mediated by trialkyl- and triphenyltin compounds. *Eur. J. Biochem.* **14**:120–126
17. Sener, A., Hutton, J.C., Kawazu, S., Boschero, A.C., Somers, G., Devis, G. 1978. The stimulus-secretion coupling

of glucose-induced insulin release. Metabolic and functional effects of NH_4^+ in rat islets. *J. Clin. Invest.* **62:**868–878

18. Tarvin, J.T., Sachs, G., Pace, C.S. 1981. Glucose-induced electrical activity in pancreatic B-cell: Modulation by pH. *Am. J. Physiol* **241:**C264–C268
19. Thomas, R.C. 1977. The role of bicarbonate, chloride and sodium ions in the regulation of intracellular pH in snail neurons. *J. Physiol. (London)* **273:**317–338
20. Trivedi, B., Danforth, W.H. 1966. Effect of pH on the kinetics of frog muscle phosphofructokinase. *J. Biol. Chem.* **241:**4110–4112
21. Vaughan-Jones, R.D. 1979. Regulation of chloride in quiescent sheep-heart Purkinje fibers studied using intracellular chloride and pH-sensitive micro-electrodes. *J. Physiol. (London)* **295:**111–137
22. Wanke, E., Carbone, E., Testa, P.L. 1979. K^+ conductance modified by a titratable group accessible to protons from the intracellular side of the squid axon membrane. *Biophys. J.* **26:**319–324

Received 4 June 1982; revised 23 September 1982